

Effect of starvation on growth, histology and ultrastructure of digestive system of juvenile red swamp crayfish (*Procambarus clarkii* Girard)

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Abstract

The present study was conducted to evaluate the effect of 20 d starvation on growth, survival, histomorphology and ultrastructure changes in the digestive system of juvenile red swamp crayfish (*Procambarus clarkii*). Juveniles were divided into two groups: a food-deprived group and a control group at 9 day after hatch (DAH). Individuals were sampled at 14, 20, 29 DAH. During the 20 d fasting period, the mean body weight and total body length of crayfish fluctuated around 10.17 mg and 8.12mm respectively, and the mortality was zero. Histomorphological changes of digestive system were observed in the food-deprived group after 20 days of starvation: the esophagus and stomach walls were thinning, the epithelium atrophied to cuboidal, nuclei were darker and smaller, and nucleolus was difficult to observe; the midgut and hindgut showed wider volume, thinning wall, atrophied epithelial and muscularis and shorter ridges; and hepatopancreas tubule lumens were wider, the lipid droplets in R-cells were smaller and less, and the quantity of typical B-cells decreased. Changes in the ultrastructure of starved crayfish were also observed: the mitochondria of midgut epithelium and R-cells were swollen and vacuolated, and the ridges of which were fractured and reduced. In addition, the electron density of cytoplasmic matrix of R-cells decreased, and the quantity of glycogen granules and lipids also decreased. Changes in the ultrastructure of B-cells were similar to those of R-cells. Though degeneration in histological structure and function of digestive organs were obvious during starvation, juvenile *P. clarkii* was able to endure a relative long-term starvation.

Keywords: *Procambarus clarkii*, Juvenile, Starvation, Histomorphology, Ultrastructure, Digestive system

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Introduction

Food shortage is a natural part of the life cycle of many aquatic animals (Sánchez-Paz *et al.*, 2006; Vinagre *et al.*, 2007). Crustaceans occasionally undergo nutritional stress due to molting or ecdysis, in which the crustaceans shed their exoskeleton to grow. In aquaculture operations, larval shrimp used as seed shrimp may experience fasting during handling, shipping and stocking (Stuck *et al.* 1996). During low- or non-feeding period, larval shrimp may change their physiological, metabolic and behavioral condition to sustain life. Changes in locomotor activity, reduction in metabolic rate, and depletion of protein, carbohydrate, glycogen and lipid reserves have been found to be associated with early stages of nutritional stress in crayfish and other crustaceans (Regnault, 1981). Long term starvation may induce several pathological changes of organs and even death in organisms (Stuck *et al.*, 1996). Bisbal and Bengtson (1995) reported that the histomorphological changes of digestive system play an important role in evaluating the nutritional condition in fish during starvation. In the last few years, several efforts have been addressed to evaluate the effect of starvation on physiological, metabolic and behavioral changes in crustaceans (Sánchez-Paz *et al.*, 2007; Matozzo *et al.*, 2011; Zhang *et al.*, 2015). Although the effect of starvation on pathological changes of organs in animals have been studied, only limited information indicating the effect of starvation on histomorphological and ultrastructure changes of digestive

system in crustaceans is available, including the hepatopancreas of *Palaemon serratus* (Papathanassiou and King, 1984), *Homarus americanus* (Anger *et al.*, 1985), *Dolops ranarum* (Tam and Avenant-Oldewage, 2009).

Red swamp crayfish *Procambarus clarkii* (Girard), a prevailing and world-wide freshwater crayfish (Cruz and Rebelo, 2007), originates from North-eastern Mexico and South-Central America. The red swamp crayfish is a commercially important shrimp species in certain countries and has been widely cultured (Henttonen and Huner, 1999). Studies on nutrition (Hammer *et al.*, 2000, Wen *et al.*, 2007) have been reported in *P. clarkii* to provide a theoretical basis to crayfish aquaculture. The effect of starvation on *P. clarkii* has also been reported. Total free amino acids and D- and L-alanine in muscle and hepatopancreas of *P. clarkii* decreased during starvation (Okama and Abe, 1998). Significant degenerations in histological structure and changes in function of digestive organs were observed in *P. clarkii* during starvation (Wei *et al.*, 2010). Guo and Zhu (1997) reported that the development of larval *P. clarkii* could be divided into three stages. The III stage larva has completely absorbed the yolk, and begins to move freely and feed, so it is reasonable to consider the development of red swamp crayfish before the third instar as larval development, whereas that from 4 to 11 stage as juvenile development.

Though as a freshwater species, the reproduction of *P. clarkii* is in autumn. Larval and juvenile *P. clarkii* often

experience starvation during the molting and juvenile development due to food shortage. How does this fish species endure the starvation to survive? The aim of the present study is to evaluate the effect of 20 days of starvation on total length, body weight, histomorphological changes of esophagus, stomach, midgut, hindgut and hepatopancreas and ultrastructure changes of hepatopancreas and midgut in the *P. clarkii* after hatch (from larva to juvenile), so as to gain insights on the connection between episodes of food shortage and digestive physiology. The results of this study would aid in the understanding of some aspects of nutrition and ecophysiology of this commercially important crayfish and/or diet supplementation regimes for the culture of this species.

Materials and methods

Culture of juvenile P. clarkii

Juvenile *P. clarkii* were bred by parent shrimp in the Fisheries Laboratory in Huazhong Agricultural University. After larvae left their mother (9 DAH), every 60 larvae were transferred into a glass tank (20cm×10cm×10cm) with a water depth of 10 cm. Larvae were separated into two groups: the food-deprived group and the control group. The crayfish in each glass tank were considered as a replicate and there were triplicates for each group. In the control group, juvenile *P. clarkii* were fed with egg yolk and commercial pelleted feed (Rudong Chengyida feed factory, Rudong Jiangsu China) which contained 32% crude protein, 3% crude lipid, 8% crude fiber, 17% crude ash, and 12.5% moisture and

were processed into 2.5 mm diameter pellets. During the 9 to 12 DAH, larvae were fed a mixture of egg yolk and commercial feed. Following 12 DAH, juvenile were merely fed with commercial feed. During the experimental period, juvenile were fed to apparent satiation twice a day (08:00 and 18:00 hours). For the food-deprived group, crayfish were totally starved during the whole experimental period. Oxygen was bubbled into the glass tanks. The temperature was monitored and kept constant at approximately 24°C, pH was 6.7 to 7.5, and a natural light-dark regime was provided.

The juvenile crayfish culture experiment lasted 29 days.

Experimental specimens

The behavior (swimming and molting) of the individuals was observed daily until the crayfish were sampled or the individuals died. The maximum number of days survived in the food-deprived group was recorded and the survival rate in each tank was calculated. Individuals were sampled and collected at 14, 20, 29 DAH in different treatment groups. For each sampling, every 10 crayfish from each group was randomly selected and batch-weighed and then the total body length of 5 crayfish was measured under a dissecting microscope (Olympus SZ2-ILST, Olympus Corporation, Tokyo Japan). Another 5 crayfish were fixed in Bouin's fixative for subsequent histological analysis. Hepatopancreas and midgut from *P. clarkii* juveniles were cut into 1mm³ pieces and prepared for transmission electron microscopy by

fixation in a mixture of 2.5% glutaraldehyde, 0.67M glucose, 0.01M sucrose, and 0.10 M sodium cacodylate buffer (pH 7.2) for 4h at 4°C.

Histological observations

The larvae and juveniles were fixed in Bouin's solution, dehydrated through a series of alcohol concentrations, cleared in xylene. Samples were then embedded in paraffin. Sagittal or transverse sections (4-6 µm thick) were obtained with an Olympus microtome (SZX7, Olympus Corporation, Tokyo Japan), stained with hematoxylin-eosin for general acidophilic and basophilic histological features. Observations by light microscopy were performed at several magnifications to describe the effect of starvation on the digestive system. The results were observed under a ZEISS microscope (Axio Imager A2, Germany) and photographed with a Nikon Optiphot compound microscope (NIS-Element BR 3.0) equipped with planachromatic objective lenses and an HFX-II photomicrographic attachment.

Ultrastructure observations

The ante-fixed specimens of hepatopancreas and midgut for transmission electron microscopy were thoroughly washed in 0.1M phosphate buffer (pH 7.2-7.4) three times, and were post-fixed for 2h with 1% osmium tetroxide. The samples were then quickly dehydrated in increasing concentrations of ethanol up to 100%, exchanged in propylene oxide, and embedded in SPI-812 resin block. Thin sections were cut in 60-80 nm by Leica UC6

ultramicrotome (Leica Camera AG, Solms Germany) and were stained with uranyl acetate followed by lead citrate. Then samples were examined in Hitachi-7650 (Hitachi Corporation, Tokyo Japan) transmission electron microscope and were photographed by Gatan832 digital imaging system (Gatan Corporation, Pleasanton USA).

Statistical analysis

Values were expressed as mean±SEM. The differences between group means were compared by independent t-test using the SPSS 19 computer program. Differences between treatments were considered significantly at $p<0.05$.

Results

The effect of starvation on crayfish growth

The growth of the total body weight of juvenile red swamp crayfish in the control group and the food-deprived group during the present experiment are shown in Fig. 1. On 29 DAH, the body weight of juveniles in the control group increased more than 7 times and the final body weight averaged at 38.75 mg, however, the body weight in the food-deprived group did not increase significantly and fluctuated at the weight of crayfish at 9 DAH (10.17 mg).

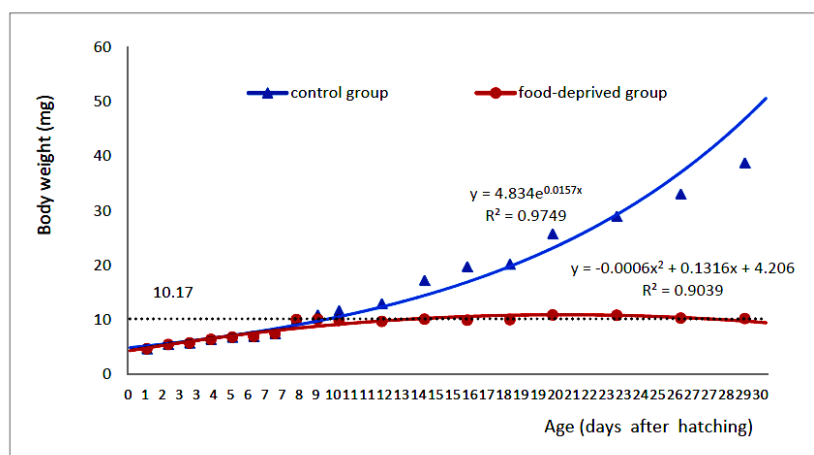


Figure 1: The effect of starvation on body weight of juvenile red swamp crayfish during the experiment.

The model of weight increase in the control group and the food-deprived group could be expressed as follows by exponential and polynomial regression (W = weight, x = days), respectively:

In the control group:

$$W \text{ (mg)} = 4.834e^{0.0157x} \quad (R^2 = 0.9749)$$

In food-deprived group:

$$W \text{ (mg)} = -0.0006x^2 + 0.1316x + 4.206 \quad (R^2 = 0.9039)$$

As shown in Fig. 2, the average body length of crayfish in the food-deprived group fluctuated at 8.12 mm during starvation, and no molting was observed. However, crayfish in the control group molted five times during the period, and the final average body length was 11.44mm. Also, juveniles in food-deprived group appeared lethargic and preferred crawling to swimming. The mortality in both groups was zero during the experiment. However, compared with the control, the food-deprived crayfish died out of water quickly during sampling.

The effect of starvation on histomorphological changes of digestive tract

As shown in Fig. 3A, the esophagus of juvenile red swamp crayfish in the control is a narrow and vertical tube connecting the mouth and cardiac stomach, which is composed of four different layers: chitinous cuticle, epithelial cells, connective tissue and muscularis. The epithelial cells of the esophagus are columnar epithelium cells. In the food-deprived group, the histomorphological structure of esophagus was not greatly damaged by 20 d starvation (29DAH). Compared to the control, the esophagus wall of the starved crayfish was thin, the columnar epithelium, atrophied to cuboidal, the nuclei were darker and smaller, and nucleoli were difficult to observe (Fig. 3B).

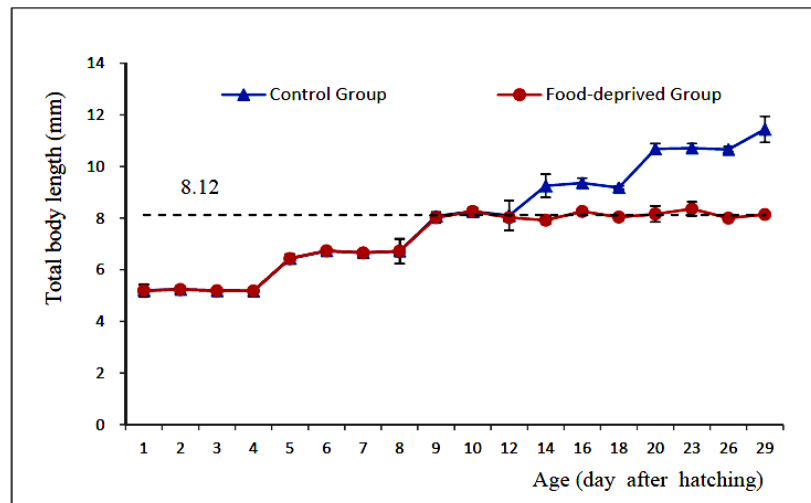


Figure 2: The effect of starvation on total body length in juvenile red swamp crayfish. The dotted line indicates length of food-deprived group was fluctuated at the value of 8.12 mm. Points represent mean \pm SEM.

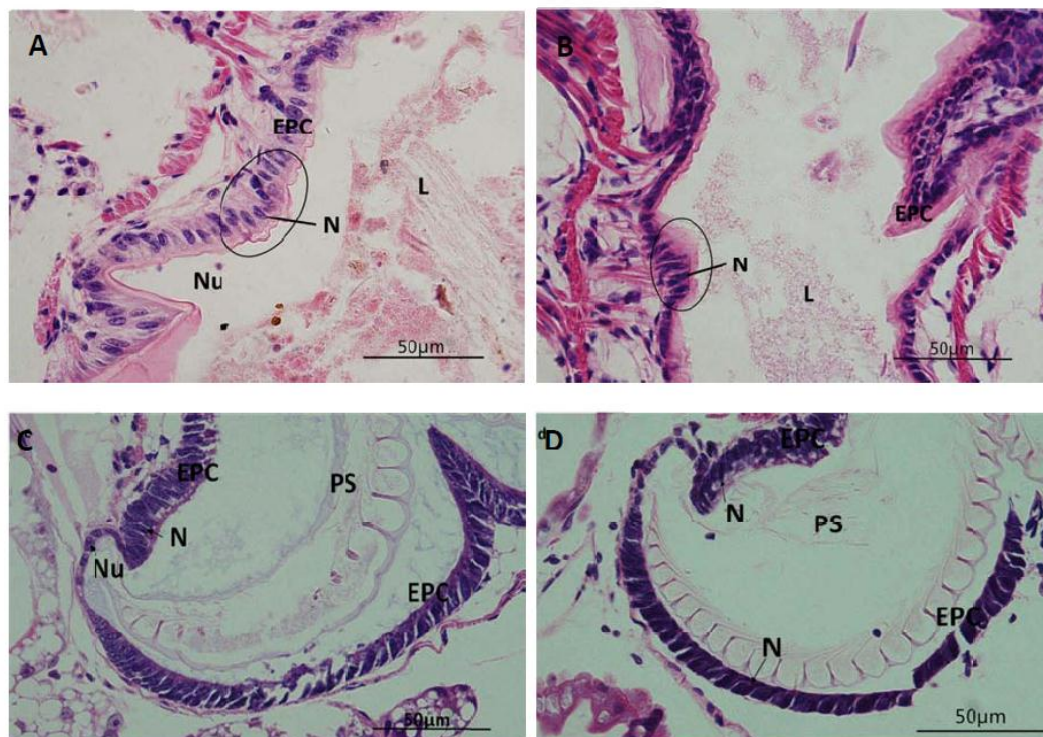


Figure 3: Histology of the esophagus and pyloric-stomach in the control and starved juvenile red swamp crayfish (Transverse sections). (A) Normal esophagus of the control group with columnar clear nuclei and nucleolus "o" is showing epithelium cells and creatine. (B) Esophagus of food-deprived crayfish after 20 days of fasting with atrophied and cuboidal epithelium "o" is showing epithelium cells and ceratine, darker and smaller nuclei, and hardly distinguishable nucleolus. (C) The pyloric-stomach in the control group with columnar epithelium and clear nuclei and nucleolus. (D) The pyloric-stomach in food-deprived crayfish with cuboidal epithelium and smaller and darker nuclei. EPC= epithelium cell, L= Lumen, N= Nuclei, Nu= nucleolus, PS= pyloric-stomach.

The histo-anatomical structure of the stomach, which is immediately posterior to the esophagus, is similar to that of the esophagus (Fig. 3C), histomorphological changes of which were also similar to esophagus after starvation, cuboidal epithelium and smaller, darker and small nuclei and unobvious nucleoli were observed in the stomach of food-deprived group (Fig. 3D).

As shown in Fig. 4A, the midgut of crayfish in the control is composed of epithelial cells, connective tissue, muscularis and tunica externa. The thick epithelium is composed of elongated columnar cells that extend from the basement membrane to the lumen, with central nuclei and a striated border at the lumen facing surface. Additionally, muscularis and epithelium together protruded into lumen to form six midgut ridges. Compared to the control group, the midgut of juveniles after 20 d fasting (29DAH) showed wider volume, thinner wall, and atrophied epithelial and muscularis, which resulted in the midgut ridges of starved crayfish to be shorter than that in the control (Fig. 4B). In addition, similar to the esophagus and stomach, the nuclei were darker and smaller, and nucleoli were difficult to observe (Fig. 4A). The height of midgut ridge was significantly ($p<0.05$) decreased by different periods of starvation, and the lowest value was found in the juveniles subjected to 20d starvation (Fig. 5). In the control group, the height of the midgut ridge increased significantly with food supply ($p<0.05$). The height of midgut ridges in starved crayfish decreased significantly ($p<0.05$)

as the starvation continued. However, no significant differences were observed in the ridge height of starved crayfish between 21DAH and 29DAH (Fig. 5). At 14DAH (5d fasting), the height of midgut ridge between the food-deprived group and the control group was not significantly different. In contrast, significant ($p<0.05$) differences were observed between the two groups at 21DAH and 29DAH (Fig. 5).

Histomorphologically, the hindgut is also composed of an epithelial layer, connective tissue, muscularis and tunica externa. As shown in Fig. 6, the effect of starvation on hindgut histology was similar to that in the midgut. After 20 days of fasting, the volume of the hindgut in food-deprived crayfish was wider, the wall of hindgut was thinner, and the epithelial layer and muscularis were atrophied, which resulted in the shorter hindgut ridges in food-deprived crayfish when compared with that in the control group. The height of the hindgut ridge was significantly influenced by different periods of starvation (Fig. 7). In the control group, the height of hindgut ridge increased significantly with food supply ($p<0.05$). However, there was a decreasing trend in the height of hindgut ridge in the starved crayfish, and the height of crayfish at 29DAH was slightly but not significantly ($p>0.05$) higher than that of the crayfish at 21DAH. Compared to the control group, the height of midgut ridge of the food-deprived group was significantly ($p<0.05$) lower after fasting for 5d or more (Fig. 7).

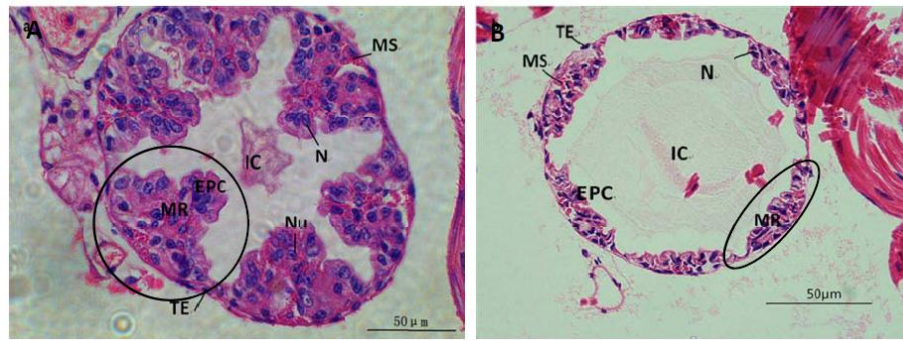


Figure 4: Histology of the midgut in control and starved juvenile red swamp crayfish (Transverse sections) at 29DAH. (A) Midgut of the control with thick epithelium and wall, clear nuclei and nucleolus. "o" is showing midgut ridge. (B) Midgut of starved crayfish with thin epithelium and wall, darker and smaller nuclei, unclear nucleolus, and atrophied midgut ridge. "o" is showing midgut ridge, EPC= epithelium cell, TE= tunica externa, IC= intestine caecum, MR= midgut ridge, MS= muscularis, N= nuclei, Nu= nucleolus.

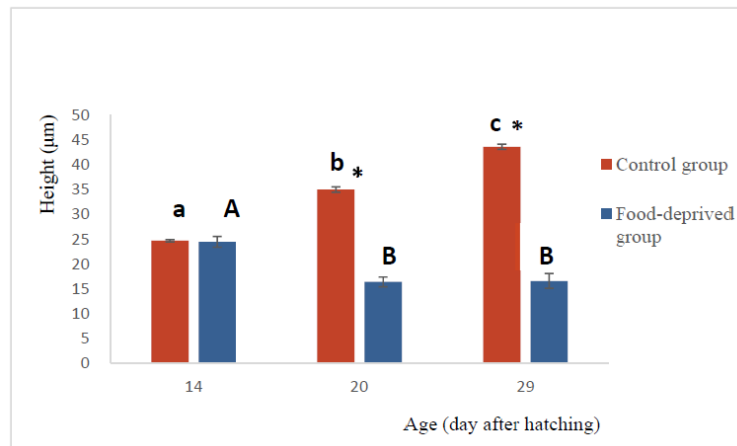


Figure 5: The height of midgut ridge in food-deprived and control crayfish at different ages. Different values of height of midgut ridges (mean±SEM, n=3) with different superscript letters were statistically significant within the same group, and with "*" were statistically significant between the control and the food-deprived group at the same age ($p<0.05$).

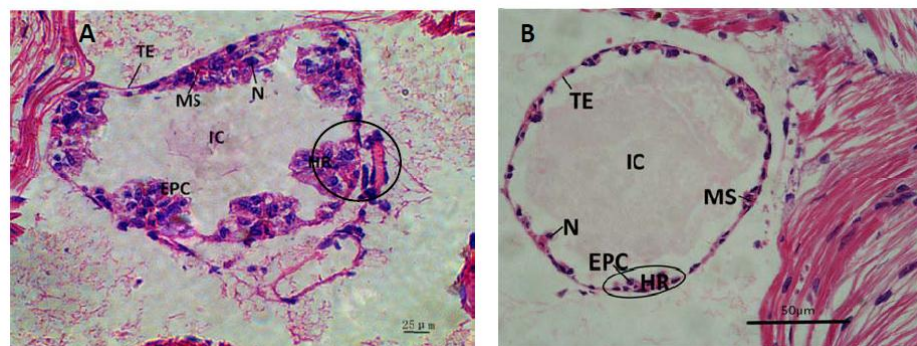


Figure 6: Histology of the hindgut in the control and starved juvenile red swamp crayfish at 30DAH (Transverse sections). (A) Hindgut in normal feeding crayfish with thick epithelium and wall, and clear nuclei and nucleolus. "o" is showing hindgut ridge. (B) Hindgut of starved crayfish with thin epithelium and wall, darker and smaller nuclei, unclear nucleolus, and atrophied hindgut ridge. "o" is showing hindgut ridge. EPC= epithelium cell, TE= tunica externa, IC = intestine caecum, HR= hindgut ridge, MS= muscularis, N= nuclei.

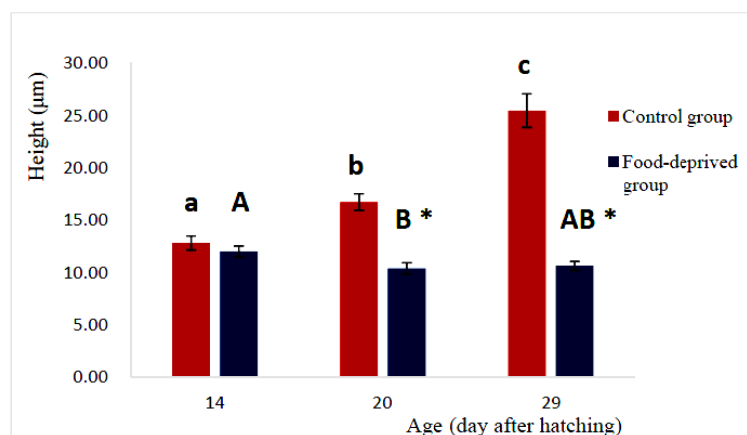


Figure 7: The height of hindgut ridge in the control and food-deprived crayfish at different ages. Different values of height of midgut ridges (mean±SEM, n=3) with different superscript letters were statistically significant within the same group, and with "*" were statistically significant between the control and the food-deprived group at the same age ($p<0.05$).

The effect of starvation on histomorphological changes of hepatopancreas

The hepatocytes of juvenile crayfish are composed of the clearly distinguishable cell-types: R (resorptive)-, F (fibrillar)-, B (blister-like)-, and E (embryonic)-cells. As shown in Fig. 8, in the fasted crayfish, the histomorphological structure of the R- and B-cells of hepatopancreas were mainly influenced by the fasting period. After 5 and 10 d of starvation, the histomorphological structure of hepatopancreas did not show any clear change, neither did the quantity of lipid droplets obviously decrease (Figs. 8A-B). However, after 20 d of fasting, hepatopancreas tubule lumens in food-deprived crayfish were wider, and the lipid droplets in R-cells became smaller and fewer in number (Fig 8D). The quantity of typical B-cells in the food-deprived group was also obviously less than that of the control after 20 days of starvation (Fig. 8C-D).

The effect of starvation on ultrastructure changes of midgut epithelium cell

As shown in Fig. 9, the ultrastructure of epithelium cells in the midgut of the control group and the food-deprived group were compared. In the control group, mitochondria and endoplasmic reticulum were the most of the organelles in the epithelial cells of the midgut wall. The epithelial cells also were rich in microvilli and different sizes of cisterna. The mitochondria were oval and densely packed with more electron density, the ridges of which were arranged in order and plainly visible (Fig. 9A). After 20 days of fasting, the microvilli height or length in the midgut of the fasted crayfish appeared to decrease, the mitochondria became large and swollen with irregularly arranged cristae, which appeared to be in the process of disintegration. Most of the rough endoplasmic reticulum in the cells of fasted crayfish showed extensive vesiculation.

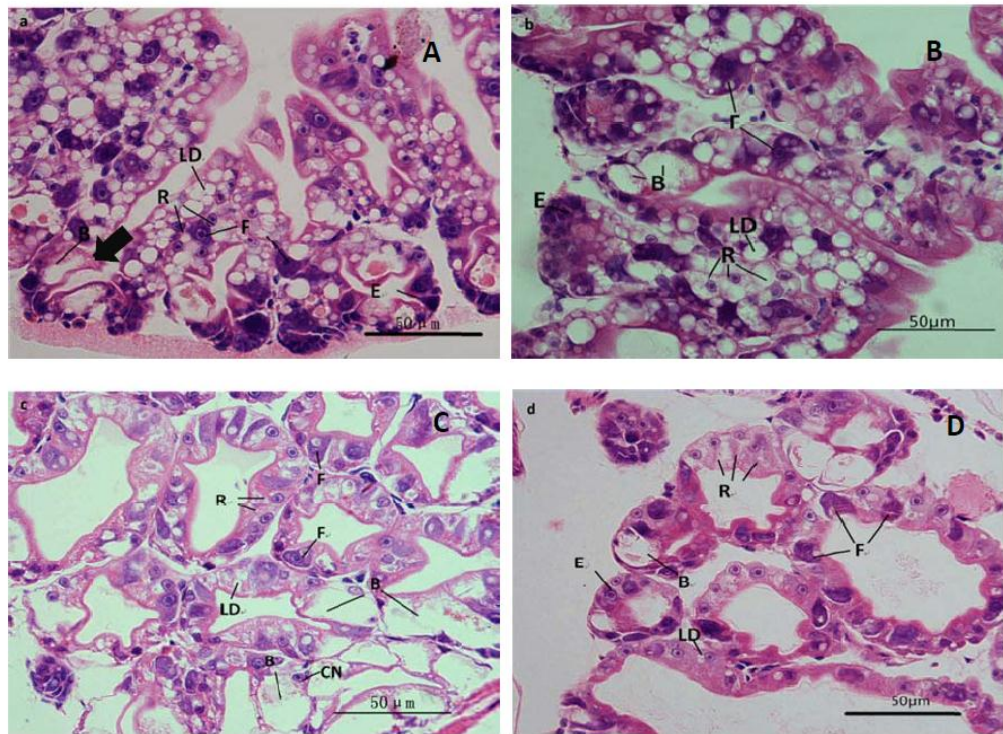


Figure 8: Histology of the hepatopancreas in the control and starved juvenile red swamp crayfish at different ages. (A) Hepatopancreas of crayfish at the 14 DAH in control group showed considerable lipid droplets, **➡** indicates a ruptured B-cell. (B) Hepatopancreas of food-deprived crayfish at 14DAH showed considerable lipid droplets. (C) Hepatopancreas of the control crayfish at 29DAH showed considerable lipid droplets in R-cell and rich B-cells. (D) Transverse sections through the shrimp of 29DAH in food-deprived group, show small and less lipid droplets and less B-cells. B= B-cell nuclei, E= E-cells, F= F-cells, LD= lipid droplet, R= R-cells.

The cisternae in food-deprived crayfish were bigger than those in the control.

The effect of starvation on ultrastructure changes of hepatopancreas

The ultrastructure of hepatopancreas cells in the control group and food-deprived group are shown in Fig. 10 and Fig. 11. In the control, the mitochondria with electron-dense matrices were spherical, and elongated in some cases. After 20 days of fasting, the mitochondria of B-cells were swollen and vacuolated, and the ridges of which were fractured, reduced and scattered. In addition, the endocytic vesicles were smaller than the

control (Fig. 10). The ultrastructure of R-cells in food-deprived group shows that the quantity of lipids decreased (Fig. 11).

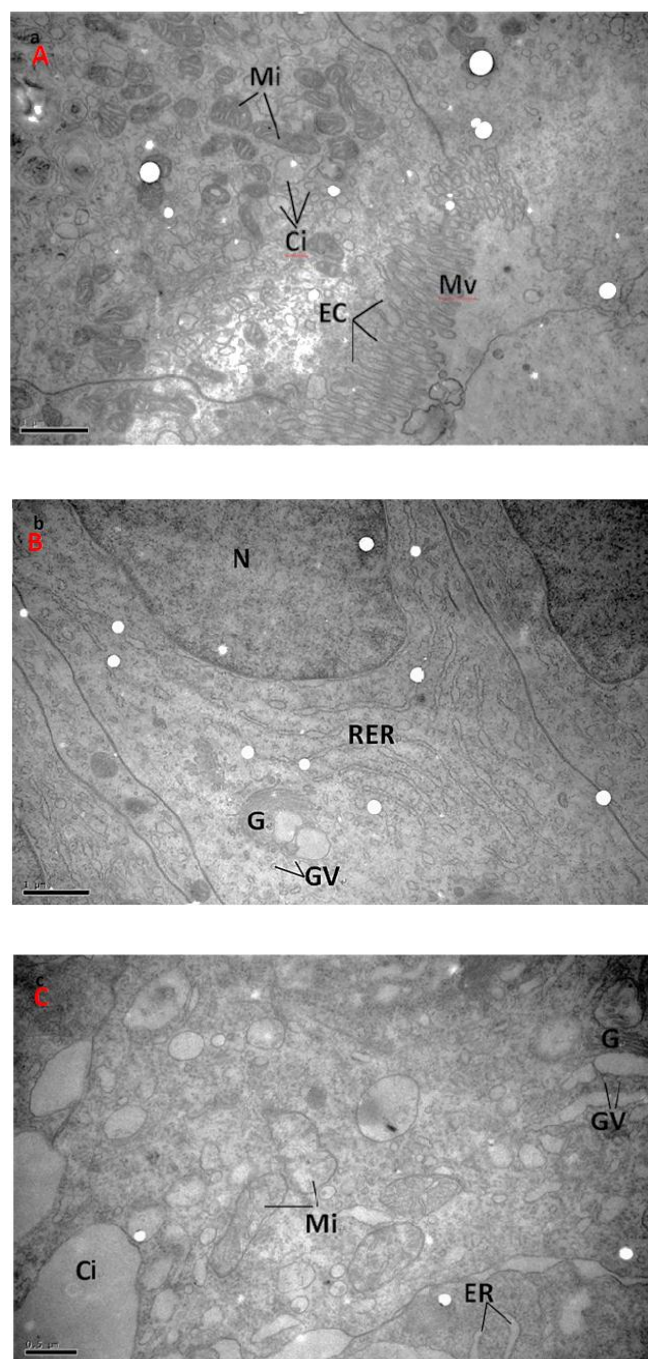


Figure 9: Ultrastructure of the midgut epithelium cell in the control and starved red swamp crayfish. (A) Midgut epithelium of the food-deprived crayfish 29DAH showed oval mitochondria which were densely packed with more electron density and the ridges of mitochondria were orderly arranged and plainly visible. (B) Midgut epithelium of the control group at 29DAH showed less mitochondria. (C) Midgut epithelium of the control group at 29DAH showed the vacuolation of swollen mitochondria, fractured ridges and bigger cisternam, which were indicated by arrow tip. Ci= cisterna, EC= endocytotic channels, ER= endoplasmic reticulum, G= golgi apparatus, GV= golgi vesicle, Mi= micrographs, Mv= microvilli, N= nuclei, PER= rough endoplasmic reticulum.

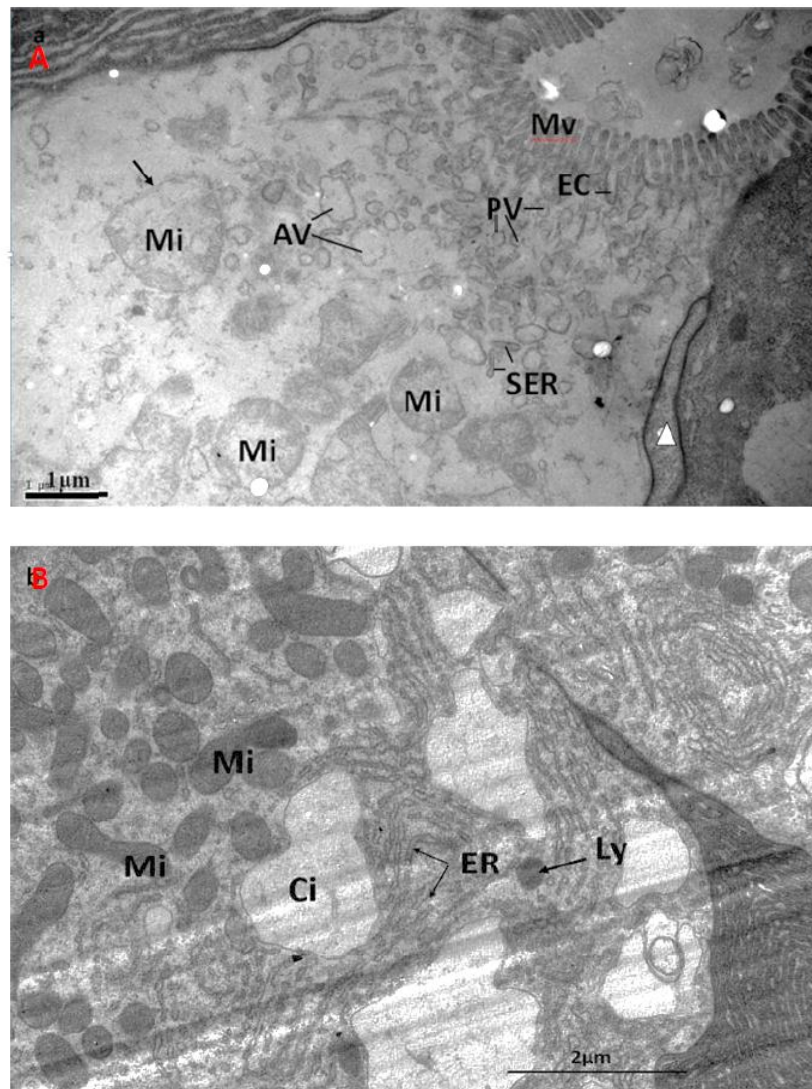


Figure 10: Ultrastructure of B-cell in the control and starved red swamp crayfish at 29DAH. (A) B-cell in food-deprived crayfish, showed vacuolation of swollen mitochondria, fractured ridges, and irregular apical vacuole. "Δ" indicates that B-cell was not close connected with neighborhood cell. **(B)** B-cell micrographs of the ultrastructure in crayfish of control group on 29DAH, showed that the mitochondria were oval and densely packed with more electron density, the ridges of it were arranged in order and plainly visible, abundant endoplasmic reticulum. AV= apical vacuol, Ci= cisterna, EC= endocytotic channels, ER= endoplasmic reticulum, Ly= lysosome, Mi= micrographs, Mv= microvilli, PV= pinocytotic vesicle, PER= rough endoplasmic reticulum.

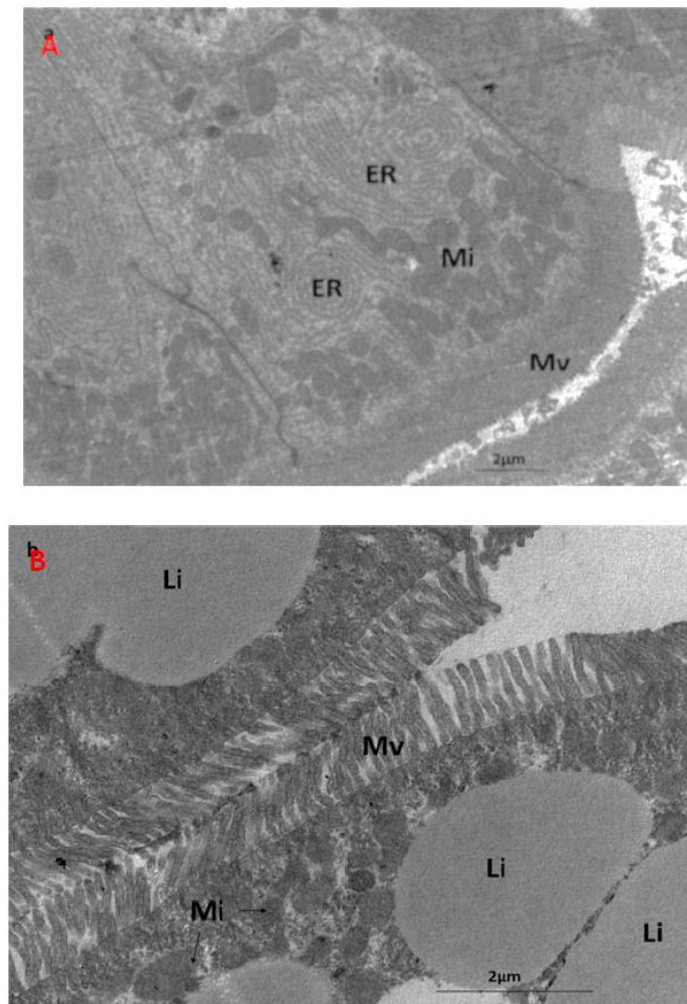


Figure 11: R-cell micrographs of the ultrastructure in the normal and starved crayfish. (A) R-cell micrographs of the ultrastructure in crayfish of food-deprived group on 29DAH, showed the few lipid. (B) R-cell micrographs of the ultrastructure in crayfish of control on 29DAH, showed that R-cell stored a lot of lipid. ER= endoplasmic reticulum, Ly= lysosome, Li= lipid, Mi= micrographs, Mv= microvilli.

Discussion

The present study showed that sufficient food supply accelerated larval and juvenile crayfish growth, which is similar to the results that higher survival and growth could be achieved by the continuous presence of food for larvae and juveniles of Atlantic sturgeon (Roustaian *et al.*, 2015). In our study, the experimental crayfish larvae of 9 DAH, which were in the third developmental stage, had basically accomplished the

development of external structures and absorbed the yolk, and began to move freely and feed, and were fully capable of living apart from the mother. Similar results were reported by Guo and Zhu (1997).

The commencement of the exogenous feeding is one of the critical stages in aquaculture of many farmed species (Roustaian *et al.*, 2015). Larval and postlarval crustaceans are difficult to endure prolonged starvation, and they

can easily reach a "point of no return" after only a few days of fasting (Anger *et al.*, 1985). Stuck and Overstreet (1994) reported that 13 to 14 d-old postlarvae of *Penaeus vannamei* (Boone) experienced 42% mortality after 10 d starvation. In contrast, adult crustaceans are generally more resistant to prolonged starvation (Stuck *et al.*, 1996). Vogt *et al.* (1985) reported that 50 d-old postlarval *Penaeus monodon* (Fabricius) were able to withstand an absence of food for a maximum of 15 d, and mortality firstly appeared after 5d of starvation. As a general response, resistance to fasting or starvation may confer adaptation to a range of marginal food conditions, including fluctuating availability of resources. In the present study, food-deprived crayfish was starved at 9 DAH and the starving experiment lasted 20 days. It is surprising that the survival rate of juvenile *P. clarkii* was 100% during the period of experiment, which showed a great capacity of tolerance to starvation conditions. Maybe this is one of the major reasons that juvenile red swamp crayfish is widely distributed in the world.

There are numerous reports on the effect of starvation on body weight in crustaceans. Several studies have shown that crustaceans usually lose weight by mobilizing accumulated endogenous energy reserves during fasting (Dawirs, 1984; Wen *et al.*, 2006; Sánchez-Paz *et al.*, 2007; Zhang *et al.*, 2009). For example, in juvenile *Eriocheir sinensis* (Milne-Edwards), body weight after 70 days of starvation was less than 50% of the value in the control (Wen *et al.*, 2006).

Similarly, in juveniles of *Fenneropenaeus chinensis* (Osbeck), 8.7, 17.9, 26.5, and 40.9 percent of wet weight loss were observed after 4, 8, 12, and 16 days of starvation, respectively (Zhang *et al.*, 2009). However, in the present study, we found that the wet weight of food-deprived crayfish was not significantly decreased during the 20 days of starvation. This is similar to the study in the Pacific white shrimp, which showed no significant effect of fasting on the total weight when shrimp were starved up to 5d (Sánchez-Paz *et al.*, 2007). During starvation, energy is derived solely from endogenous resources, and tissues are lost due to catabolic activities (Stuck *et al.*, 1996). Because the volume of a crustacean becomes fixed by its exoskeleton (Stuck *et al.*, 1996), to maintain the necessary body volume and internal turgidity, the lost tissue mass must be replaced by water (Wilcox and Jeffries, 1976). And this may partly explain why juvenile *P. clarkii* have great capacity of tolerance to starvation conditions.

In this study, no molting was observed in food-deprived group during the fasting period. Similar results have been reported in *Carcinus maenas* (Linnaeus) (Dawirs, 1984), *Thenus* sp. *phyllosomas* (Mikami *et al.*, 1995), and *Penaeus vannamei* (Sánchez-Paz *et al.*, 2003). It has been reported that the secretory rate of molting hormones in the individuals which lacked food was lower than that of the normal individuals (Anger and Spindler, 1987). Cuzon reported that glycogen in the hepatopancreas is an important precursor

for chitin synthesis (Cuzon *et al.*, 2000). Therefore, these results may suggest that the molting activity of juvenile *P. clarkii* could be suppressed by the undernutrition condition.

During the food deprivation period, the histological and ultrastructural data may be more informative than changes in the digestive organ size (Secor *et al.*, 2000). Many studies have reported the histological and ultrastructural changes of intestine in fish due to starvation (Bisbal and Bengtson, 1995; Gisbert and Doroshov, 2003). In the present study, the sensitive organs in response to the starvation also were midgut and hindgut. The thin wall, wide volume, shorter gut ridges, atrophied epithelial layer and muscularis were observed in the intestine of fasted crayfish. Statistically, the height of midgut and hindgut ridges in starved crayfish decreased significantly after 10 d starvation compared to that in the control. However, no significant difference in the ridge height of starved crayfish between 21DAH and 29DAH was observed, which indicated that juvenile crayfish can endure fasting to a certain extent in long starvation. Interestingly, at 14DAH, the height of midgut and hindgut ridge was not significantly different between the food-deprived group and the control group. The possible reason is that the I-II stage larval crayfish stored lipid and glycogen by absorbing yolk (Unpublished data). When the larval ontogeny is into the III stage (9DAH-12DAH), the juvenile red swamp crayfish store abundant substances of nutrition to maintain metabolism to

endure starvation. Changes in the ultrastructure of the midgut epithelial cells of starved crayfish included the bigger and swollen mitochondria with irregularly arranged and even fractured cristae. Similar results have been reported in *Daphnia magna straus* (Elendt and Storch, 1990) and *Dolops ranarum* (Stuhlmann) (Tam and Avenant-Oldewage, 2009). All of the responses implied that starvation had dramatic effects on the structure of intestinal epithelium and muscularis and the digestive function, and decreased the metabolic activity in the epithelial cells of the intestine. When energy substances reserves were exhausted, the body tissues of the starved animals were catabolized, which caused a progressive degeneration of the digestive tract and accessory organs and down-regulated the structure and function of the digestive system to conserve energy (Zeng *et al.*, 2012). There are physiological differences between the midguts and the hindguts of decapod crustaceans (To *et al.*, 2004). The luminal epithelium cells of the midgut display both absorptive and secretory features (Jong-Moreau *et al.*, 2000). Additionally, during starvation, the cells lost their ability to control their osmotic balance (Storch and Anger, 1983; Elendt and Storch, 1990), which caused the swollen and vacuolated mitochondria with fractured ridges. As the intestine is a sensitive organ in response to starvation, the intestinal folds height can be used as a reliable indicator of starvation or sub-optimal feeding in rearing of juvenile red swamp crayfish.

Hepatopancreas is a vital organ

involved in excretion, molting, lipid and carbohydrate metabolism, and diverse metabolic activities, including synthesis and secretion of digestive enzymes, absorption of nutrients, synthesis of plasma proteins such as hemocyanin and lipoproteins (oxygen and lipid transporters and components of the defense system) and storage of energy reserves (Sánchez-Paz *et al.*, 2007). Several studies reported that hepatopancreas is mainly composed of four types of cells: E-, F-, R-, B-cells (Al-Mohanna and Nott, 1986; Al-Mohanna and Nott, 1987; Vogt, 1994). In the present study, the fine structure of R-cell and of B-cell of *P. clarkii* was mainly affected by starvation. R-cells execute the intestinal and hepatic functions including absorption and catabolism of nutrients, delivery of metabolites to other organs, storage of reserves for periods of molting, reproduction and starvation, and detoxification of heavy metals (Vogt, 1994). Our results on the decreased quantity of lipid droplets in the R-cells supported this. Several studies also reported that the R-cells showed conspicuous and dramatic changes during starvation (Vogt *et al.*, 1985; Elendt and Storch, 1990). The function of B-cells is intracellular absorption, assimilation and degradation (Al-Mohanna and Nott, 1986; Vogt, 1994). The typical B-cell has a single large vacuole which compresses the cytoplasm to a thin marginal rim. The vacuoles contain dense spherules rich in digested material (Al-Mohanna and Nott, 1986). Papathanassiou and King (1984) reported fewer B-cells were present in

the tubule of starved *Palaemon serratus* (Pennant). In the present study, we also found that the number of typical B-cells decreased obviously in the food-deprived group at 29DAH. The possible reason is that there was little digested material in the central vacuole, so that we cannot observe the typical B-cell. In addition, the mitochondria in B-cells were large and swollen and the basal endoplasmic reticulum system was poorly developed or absent after crayfish were long-term fasted, which suggest that the function and metabolism of B-cells were adversely affected by starvation.

In conclusion, this study has yielded some original data on the effect of starvation on the digestive system of the juvenile *P. clarkii*. Starvation limited the growth of juvenile *P. clarkii*, and resulted in histomorphological and ultrastructure changes of digestive system, especially affecting the epithelium cells in the digestive tract and R- and B-cells in hepatopancreas. This study indicates that juvenile *P. clarkii* have the ability to withstand a relative long-time starvation, but adequate feeding is necessary to ensure the success of commercial seedling. The knowledge derived from the understanding of starvation resistance and the corresponding of histomorphological and ultrastructure changes in *P. clarkii* will be useful in the design of feeding regimes in this species.

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